RESEARCH PROPOSAL

TITLED

" Evaluation of Synergistic Effects of *Moringa oleifera* Leaf Extracts And Tropically Used Antibiotics Against Bacterial Pathogens "

Submitted to

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1. Introduction

Antimicrobial resistance (AMR) has emerged as a global health crisis, threatening the efficacy of antibiotics and increasing morbidity, mortality, and healthcare costs. The World Health Organization (WHO) identifies AMR as one of the top ten global public health threats, emphasizing the urgent need for innovative antimicrobial strategies (Ibrahim et al., 2022). Because the development of new antibiotics is slow and expensive, researchers are increasingly investigating synergistic combinations of natural products and conventional antibiotics as a means to restore or enhance antimicrobial activity (Rangel-Vega et al., 2022).

Moringa oleifera Lam., commonly known as the drumstick tree, is a medicinal plant rich in bioactive compounds such as flavonoids, phenolics, alkaloids, tannins, and glucosinolates. Numerous studies report the antimicrobial activity of *M. oleifera* leaf extracts against diverse bacterial species, including *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* (El-Sherbiny et al., 2024). The potency of the extract depends on the solvent used, with ethanol and methanol extracts typically exhibiting higher antibacterial activity due to their ability to extract phenolic and flavonoid compounds (Aslam et al., 2021).

Recent research demonstrates that plant extracts may potentiate antibiotic activity by enhancing membrane permeability, inhibiting efflux pumps, or interfering with bacterial enzyme systems (Rangel-Vega et al., 2022). Synergy between *M. oleifera* extracts and antibiotics has been documented, particularly against resistant *S. aureus* strains (Vankwani et al., 2022). However, most previous studies evaluated only one type of extract (e.g., methanol) or focused primarily on Gram-positive bacteria, with limited comparative evaluation across solvent types and Gram-negative species.

Therefore, this study aims to evaluate the synergistic effects between *M. oleifera* leaf extracts prepared with different solvents and selected antibiotics against Gram-positive and Gram-negative bacteria. This work will provide insight into the potential of *M. oleifera* as an antibiotic-adjuvant plant and contribute to strategies combatting the growing challenge of antimicrobial resistance.

2. Literature Review

2.1 Antimicrobial activity of *Moringa oleifera*

Several recent studies (2021–2025) have confirmed the antimicrobial potential of *M. oleifera* leaves. Aslam et al. (2021) demonstrated that ethanolic leaf extracts exhibited strong antibacterial activity against *S. aureus* and *E. coli*, attributing the potency to high phenolic and flavonoid content. Similarly, El-Sherbiny et al. (2024) quantified the phytochemicals in *M. oleifera* and showed that both methanol and ethanol extracts had broad antibacterial activity, correlating antimicrobial effect with phenolic composition.

2.2 Factors influencing antimicrobial efficacy

Solvent polarity greatly affects extract composition. Ethanol and methanol typically yield stronger antimicrobial effects than water due to extraction of lipophilic phytochemicals (Abiola et al., 2023). Extraction method (maceration, Soxhlet, or ultrasound) also influences yield and antimicrobial potency.

2.3 Synergistic activity of plant extracts and antibiotics

Plant–antibiotic synergy is increasingly studied as a strategy to overcome resistance. Rangel-Vega et al. (2022) reviewed multiple plant species and concluded that phytochemicals often work synergistically with antibiotics by inhibiting efflux pumps, damaging bacterial membranes, or inhibiting β-lactamase enzymes.

2.4 Synergistic effects of *Moringa oleifera* with antibiotics

Vankwani et al. (2022) found strong synergy between methanolic *M. oleifera* stem-bark extract and ampicillin against methicillin-resistant *Staphylococcus aureus* (MRSA), reducing the MIC of ampicillin significantly and demonstrating bactericidal synergy in time-kill assays. In another study, Ahmed et al. (2023) reported that *M. oleifera* ethanolic leaf extracts enhanced the efficacy of gentamicin against both Gram-positive and Gram-negative clinical isolates.

Despite these findings, few studies have systematically compared synergy using **multiple solvent extracts** across **both Gram-positive and Gram-negative bacteria**. Most synergy studies used methanol extract only or focused solely on Gram-positive bacteria. This gap highlights the need for a broader, solvent-comparative synergy assessment.

3. Objectives

General Objective

Evaluation of Synergistic Effects of *Moringa oleifera* Leaf Extracts and Tropically Used Antibiotics Against Bacterial Pathogens

Specific Objectives.

1. To prepare and characterize Moringa oleifera leaf extracts using appropriate extraction methods.

2. To evaluate the antibacterial activity of Moringa oleifera leaf extracts against selected bacterial pathogens.

3. To assess the antibacterial effectiveness of commonly used tropical antibiotics against the same bacterial isolates.

4. To investigate the synergistic effects between Moringa oleifera leaf extracts and selected antibiotics using standard in vitro synergy testing methods.

4. Materials and Methods

4.1 Plant Collection and Extraction

Fresh *Moringa oleifera* leaves will be collected, washed, shade-dried, powdered, and extracted using three solvents:

- Distilled water (aqueous)
- 70% ethanol
- Methanol

Extraction will be performed using cold maceration (48 h), followed by filtration and solvent evaporation using a rotary evaporator (40°C). Extracts will be stored at –20°C.

4.2 Microbial Strains

- *Staphylococcus aureus*
- *Enterococcus faecalis*
- *Escherichia coli*
- *Pseudomonas aeruginosa*

4.3 Antibiotics Tested

- Ampicillin (β-lactam)
- Gentamicin (aminoglycoside)
- Ciprofloxacin (fluoroquinolone)
- Any other available antibiotics

4.4 MIC Determination

Minimum inhibitory concentrations (MICs) for extracts and antibiotics will be determined using CLSI-adapted broth microdilution assays in 96-well microplates.

4.5 Checkerboard Synergy Assay

Two-dimensional serial dilutions of extract and antibiotic will be combined.
FIC Index = FIC(A) + FIC(B):

- Synergy ≤ 0.5
- Additive/indifferent 0.5–4.0
- Antagonism > 4.0

4.6 Time-Kill Assay

Synergistic combinations (FICI ≤ 0.5) will be tested at 0, 2, 4, 8, and 24 hours.
Synergy = ≥2 log10 CFU/mL reduction vs. most active single agent.

4.7 Mechanism Screening

- Membrane leakage assay (protein and nucleic acid leakage)
- Efflux pump inhibition (EtBr accumulation assay)

5. Expected Outcomes

1. Ethanolic and methanolic extracts will show stronger intrinsic antibacterial activity compared with aqueous extract.
2. At least one extract–antibiotic pair is expected to demonstrate clear synergy (FICI ≤ 0.5), particularly with ampicillin or gentamicin.
3. Synergistic combinations will show enhanced bactericidal activity in time-kill curves.
4. Mechanistic assays will indicate increased membrane permeability or efflux inhibition.

6. Significance of the Study

- Provides scientifically grounded evidence for using *M. oleifera* as an antibiotic-potentiating natural agent.
- Offers an affordable strategy for enhancing antibiotic efficacy.
- Helps address the global challenge of antimicrobial resistance.
- Opens opportunities for future phytochemical isolation and drug development.

7. References

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